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A STUDY OF THE ASCORBIC ACID REQUIREMENTS OF CHILDREN OF EARLY SCHOOL AGE

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS
AND
HOUSEHOLD ADMINISTRATION

1942

By
VIVIAN M. ROBERTS

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A STUDY OF THE ASCORBIC ACID REQUIREMENTS OF CHILDREN OF EARLY SCHOOL AGE [1]

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THREE FIGURES

(Received for publication February 18, 1942)

The amount of ascorbic acid required for normal nutrition is not, as yet, well established. More detailed studies are needed at all age levels. Standards for the infant have been estimated, for the most part, either by determining the amount of vitamin C it takes to prevent the development of infantile scurvy, or by comparing the blood levels of breast-fed and artificially-fed infants with the relative amounts of ascorbic acid provided by the milk. While well-controlled, quantitative studies have been made on preschool children and adults, investigations on the school-age child have been principally of the survey type. In such studies the vitamin C content of the diet is only estimated, usually by dietary records or by inventory methods. This study was undertaken with the view of contributing quantitative data on the vitamin C requirements of children of early school age.

EXPERIMENTAL

Selection of subjects. The subjects of this study were five children, two girls and three boys, between the ages of 7 and 12 years, living in a home for crippled and convalescent children. This institution was chosen because living conditions were such that it was possible to control all phases of the study. Of the children selected three had been under treatment for malnutrition, one for clubfoot, and one for rheumatic heart.

[1] This research was aided by the Talcott fellowship granted by Rockford College and the Omicron Nu fellowship awarded by the American Home Economics Association.

Presented at annual meeting of American Institute of Nutrition, April 1, 1942.

In all cases treatment had been successful, and the children were judged by the physician to be in a normal state of health. They were being retained in the institution until home conditions were favorable for their dismissal. The daily routine and activity of these children were comparable to that in any home. The children attended school regularly and played outside at recess and after school.

The physical status of the subjects is summarized in table 1. It is noted that all were of approximately average weight, or above, and that all gained substantial amounts during the

TABLE 1

The physical status of subjects.

SUBJECT	AGE		SEX	CONDITION FROM WHICH RECOVERED	BODY WEIGHT	HEIGHT	PER CENT OVER- OR UNDER-WEIGHT [1]	GAIN IN WEIGHT DURING STUDY
	yrs.	*mos.*			*kg.*	*cm.*		*kg.*
M. G.	12	6	F.	Malnutrition	33.7	142.5	— 6	1.5
P. T.	11	10	M.	Rheumatic heart	43.6	147.5	+ 13	1.8
D. S.	10	3	F.	Malnutrition	31.0	138.0	— 3	2.5
R. M.	9	6	M.	Malnutrition	39.1	141.8	+ 12	2.3
L. M.	7	9	M.	Clubfoot	27.3	126.2	+ 4	2.2

[1] As judged by the Baldwin-Wood tables.

period of the study. The one who had had rheumatic heart gained 1.8 kg. in the 3 months. Weekly medical checks showed his heart condition to be completely controlled.

General plan of the study. The original plan for the study was to follow the saturation method used by Belser, Hauck and Storvick ('39) and to include, in addition, the determination of blood ascorbic acid. Since the initial blood levels of the children to be used in the study were found to be low (0.45 to 0.59 mg. per 100 cc.) it seemed wise to follow the changes in the blood levels as the intake was gradually increased, rather than to saturate the subjects at the beginning. The vitamin C content of the institutional diet was determined, therefore, and this amount used as the starting point for the investigation. To a basal diet providing 15 mg. of vitamin C, supplements of crystalline ascorbic acid [2] were added daily.

[2] Appreciation is extended to Hoffman-La Roche, Inc., Nutley, New Jersey, for furnishing the crystalline ascorbic acid.

The investigation included eight experimental periods of 7 days each. During these periods the supplements of ascorbic acid ranged from 40 to 130 mg. Starting with 40 mg. the daily supplement was increased 20 mg. each week until the blood levels remained practically constant with the increased intake. Thereafter the supplements were gradually decreased until the 50 mg. level was reached for four subjects, and 90 mg. for the fifth.

The amounts of reduced ascorbic acid in the urine and blood plasma were determined on each level of intake. At the end of each period a test dose of 300 mg. vitamin C was given. The amount excreted in the urine during the 24-hour period following this test dose was used as one criterion for judging the state of saturation of each subject. Blood levels, urinary "resting level" and "retention values" are other criteria employed in the final analyses.

Dietary control. Chemical analysis of the institutional diet showed that the vitamin C was provided, for the most part, by a small glass of fruit juice, one serving of fresh fruit, and a quart of raw milk. The rest of the food furnished little ascorbic acid, due probably to the methods of preparation employed. Lettuce and cabbage, for example, were shredded 5 or 6 hours before serving, and most vegetables were overcooked. Potatoes stood in water both before and after cooking. They were then well aerated in an electric mixer and kept on the steam table for a considerable time. As a result all the vitamin C was destroyed.

The diet, on the whole, met the usual standards for adequacy with the possible exception of thiamine and vitamin C. The low blood levels of ascorbic acid indicated a shortage of this vitamin, even though the diet provided an average of 50 mg. daily.

Few changes were necessary, then, in setting up the experimental diet. Fruit juices and raw fruits and vegetables were eliminated and replaced by canned fruits, juices, and vegetables of low vitamin C content. The basic diet which was planned to furnish 15 mg. ascorbic acid, was made up from the foods listed in table 2. The weighed foods were always given in the same amount, with the exception of the milk

which varied from 750 to 1000 gm., depending upon the vitamin C content of the rest of the diet.

Collection of urine. For quantitative work Todhunter and Fatzer ('40) and Richardson and Mayfield ('40) have pointed out the necessity of using 24-hour urinary excretions. During this investigation the daily urinary output was collected and analyzed for 3 or 4 days on each level of intake. In addition, analyses were made on the 24-hour collection following each test dose.

To preserve the ascorbic acid in the urine 5 N sulphuric acid and 8-hydroxyquinoline were used as suggested by Sendroy and Miller ('39). By using brown bottles for the urine, acidifying the samples immediately, and keeping them in the refrigerator, losses were kept within 2%.

TABLE 2

Foods used in the basal diet with amount of ascorbic acid contributed by each food.

FOOD	AMOUNT	ASCORBIC ACID	FOOD	AMOUNT	ASCORBIC ACID
Foods weighed	*gm.*	*mg.*		*gm.*	*mg.*
Fruit juices			Vegetables, canned		
Peach	120	0.7	Carrots	60	0.3
Pear	120	0.4	Beets	70	3.0
Plum	120	0.3	Beans, green	60	1.8
Apple	120	1.2	Beans, Lima	60	0.4
Dried fruit (stewed)			Potatoes, mashed	110	..
Apricots	80	2.9	Milk, raw	750–1000	7.5–12.0
Peaches	80	2.3	Cocoa	200	0.5
Pears	80	0.3	Cod liver oil	1 tsp.	..
Apples	80	0.2			
Raisins	80	0.2	*Foods served ad lib.*		
Prunes	80	0.3	Meats	daily	
Canned fruit			Beef		
Pear, Bartlett	80	0.8	Veal		
Juice	20	0.3	Lamb		
Cherries			Bacon		
(Royal Ann)	80	2.2	Chicken		
Juice	30	1.8	Eggs	4–6 times per week	
Plums (Lombard)	60	0.6			
Juice	30	0.4	Cereals	daily	
Grapes (white)	60	1.2	Bread	daily	
Juice	30	0.8			
Apple Sauce	100	0.7			

Chemical methods. Because of the impossibility of taking the photoelectric colorimeter to the institution it was necessary to use the titration method for determining ascorbic acid. Preliminary tests showed, however, that by the addition of a buffer to the acidified urine the rapid fading of the end-point was prevented and the results checked within 2% of the values obtained with the photoelectric colorimeter. The 2,6-dichlorophenolindophenol used as an indicator was standardized daily by the procedure of Menaker and Guerrant ('38). For food and urine analyses the methods of Bessey and King ('33) were used. The micro-technique with the Farmer and Abt apparatus ('36) was employed for all blood determinations. Fasting blood plasma separated from capillary blood was used, special care being taken to prevent any hemolysis.

All solutions were prepared with water redistilled in glass. A fluorescent daylight lamp was used as a source of light for titrating.

RESULTS AND DISCUSSION

As a basis for interpreting the results from these blood and excretion studies in terms of the requirements, three criteria have been used. These are based upon (1) the "retention" or "utilization" of ascorbic acid, (2) the response to a 300-mg. test dose, and (3) the concentration of ascorbic acid in the blood. Blood levels above 0.7 mg. per 100 cc., the level recommended as normal by Goldsmith and Ellinger ('39) and Wortis et al. ('38), have been used in this study to indicate a satisfactory state of nutrition in respect to vitamin C.

In addition to these three indices for estimating the ascorbic acid requirements, other methods are included in the discussion. These will provide a means of comparing the results with other reports found in the literature. Urinary "resting level," "saturation" as interpreted by Belser, Hauck and Storvick ('39) and blood levels higher than 0.7 mg. per 100 cc. have been included.

"Retention values." One method of interpreting the results from urinary excretion studies in terms of the requirements is based upon the "retention value," that is, the difference between the intake of ascorbic acid and that excreted in the urine. With preschool children, Hathaway and Meyer ('41)

demonstrated the constancy with which the body utilized ascorbic acid. They found the mean "retention" for four children to be 23 mg. on intakes ranging from 31 to 50 mg. This represented 74% of the intake at the 31 mg. level, the minimum intake on which maximum "retention" was obtained.

The tendency for the body to "retain" a nearly constant amount of ascorbic acid was observed with the subjects on this study. As shown in table 3 there is little variation in the

TABLE 3

Average daily urinary excretion and average "retention" of ascorbic acid on various levels of intake.

PERIOD	1	2	3	4	5	6	7	8	6	7	8	AVERAGE AND S. D.
Intake—mg.	55	75	95	115	135	105	85	65	145	125	105	
"Excretion" *mg.*												
M. G.	19	32	51	70	80	62	40	37				
P. T.	11	26	37	69	86	60	39	25				
D. S.	19	32	55	80	95	65	47	34				
R. M.	12	29	49	75	95	62	48	25				
L. M.	21	44	54	67	100				106	88	79	
Average	16	33	49	72	91	62	42	30				
"Retention"[1] *mg.*												
M. G.	36	43	44	45	55	43	45	28				42 ± 7
P. T.	44	49	58	46	49	45	46	40				47 ± 5
D. S.	36	43	40	35	40	40	38	31				38 ± 3
R. M.	43	46	46	40	40	43	37	40				42 ± 3
L. M.	34	31	41	38	35				39	37	26	35 ± 5
Average	39	42	46	43	43	43	43	35				

[1] Value does not include the two 24-hour specimen following the test dose

amounts "retained" by each individual on the different levels of intake tested. The values appear to be related to the weight of the children rather than to the age. They vary from 35 ± 5 mg. for L. M., the smallest subject, to 47 ± 5 mg. for P. T., the largest.

This relationship of intake to excretion and "retention" is further illustrated in figure 2. The daily urinary excretion of ascorbic acid varies in direct proportion to the intake. It is apparent, however, that the amount "retained" varies but

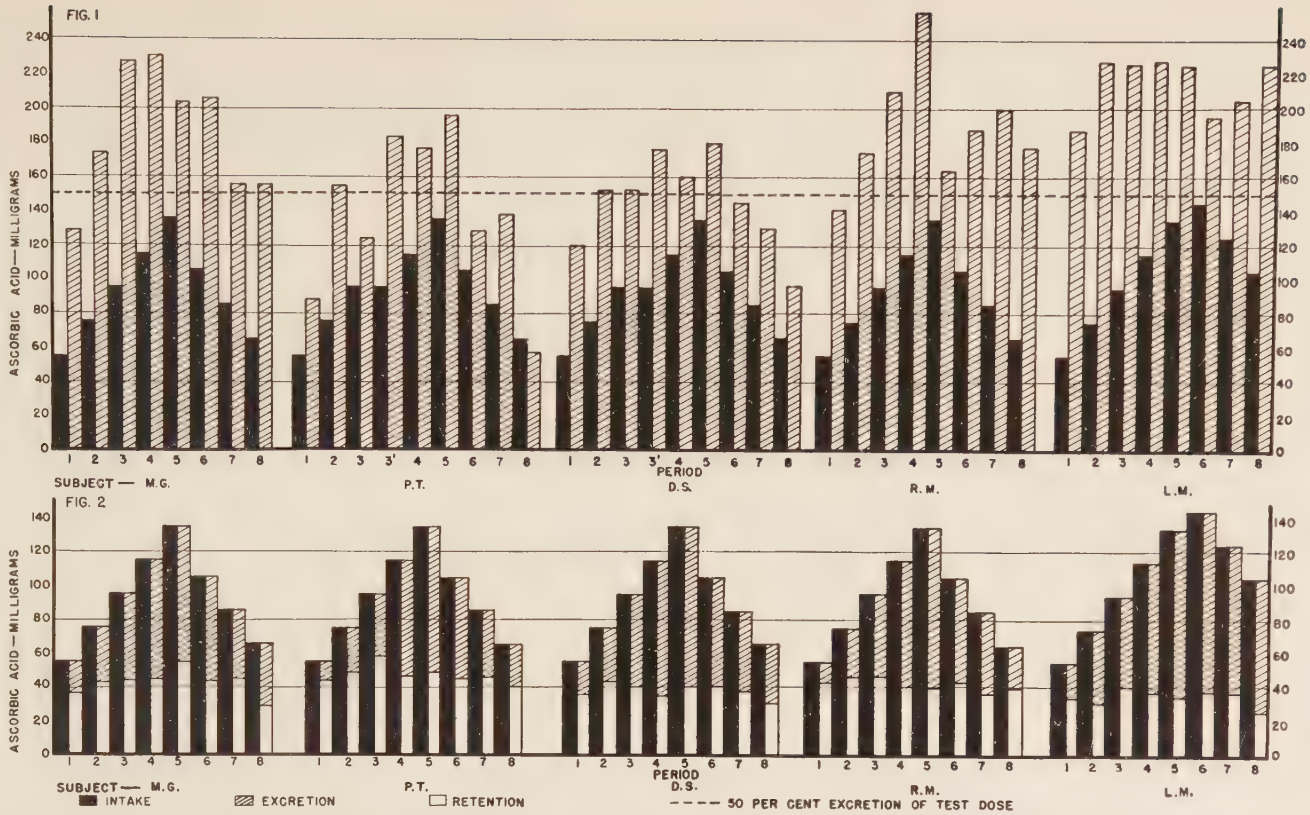

Fig. 1 24-hour excretion of ascorbic acid in response to a 300-mg. test dose following various levels of ascorbic acid intake. The black bars represent the previous intake, and the striped bars, the excretion after the test dose. 50% of the test dose is indicated by the broken line at the 150 mg. level.

Fig. 2 Average daily excretion and "retention" of ascorbic acid on various levels of ascorbic acid intake. The black bars represent the daily intake; the striped bars, the excretion; and the white bars, the "retention."

little. The lowest intake on which each subject "retained" an amount of ascorbic acid equivalent to the average "retention value" ranges from 55 mg. for two of the children to 75 mg. for the other three. The "retention" at these levels of intake represents 57 to 77% of the ascorbic acid ingested.

"*Resting level.*" The "resting level" or amount excreted daily in the urine has been used as another index for measuring the state of vitamin C nutrition. Van Eekelen and associates ('37) considered a "resting level" of 40 mg. indicative of tissue saturation. The average daily excretions for the five subjects on this study are reported in table 3. The ascorbic acid in the urine varied directly in proportion to the intake. The average excretion increased from 16 mg. on an intake of 55 mg. of vitamin C to 91 mg. when 135 mg. was ingested. An output of 40 mg. resulted from an intake of 75 mg. for one subject and 85 mg. for the other four.

Response to a test dose. A more widely accepted measure of vitamin C nutrition is the so-called "saturation test." By this method a large quantity of ascorbic acid is administered — orally, subcutaneously, or intravenously — and the amount excreted in the urine determined. A large percentage return of the test dose within 24 hours has been interpreted as indicating saturation. The exact percentage used by different workers, however, varies in respect to the size of the test dose given, the method of administration, and the length of the collection period. For this study a 300-mg. test dose of ascorbic acid was given orally, and a 50% return in the urine during the subsequent 24-hour period was used as the criterion for judging saturation.

The relationship between the intake and the response to the test dose is illustrated in figure 1. The youngest subject, L. M., returned more than 50% of the test dose on the lowest level of intake, that is, 55 mg. On all other levels he remained saturated. Sixty-five milligrams were required by M. G. and R. M. to produce the same response. They, too, remained saturated on intakes above 65 mg. The results for P. T. and D. S., however, were variable. In each case 75 mg. was the

lowest intake upon which 50% of the test dose was excreted. It will be noted that P. T. excreted less of the test dose on the 95 mg. level than on the 75 mg. intake. Upon repeating the test, the percentage return was greater. Similar variations have been observed in studies on adults (Belser, Hauck and Storvick, '37; Todhunter and Robbins, '40; and Fincke and Landquist, '42). The figures from the present study are in agreement with the findings of Koch ('41). With children of early school age she found that her subjects required 52 to 72 mg. of ascorbic acid before they excreted 50% of a 300 mg. test dose.

In figure 1 it is shown, also, that P. T. and D. S. always excreted less of the test dose than the others. The procedure followed by Belser, Hauck and Storvick ('39) rules out to some extent these differences due to individual variations. In general, they completely saturate the subject and then determine his individual response to the test dose. The value thus obtained is used to indicate his saturation response. It is questionable whether the data from the present study may be interpreted on the basis of this technique inasmuch as the subjects were not saturated at the beginning. One may assume, however, that they were all saturated at the three highest levels of intake, for the ascorbic acid level of the blood remained constant. With this interpretation, where each child sets his own standard for response to the test dose, three of the subjects required the same amount of vitamin C for saturation as was needed when the criterion consisted of 50% return of the test dose: that is, P. T., D. S., and R. M. required an intake of 75, 75, and 65 mg., respectively, to produce saturation. For the other two children the results were higher; 95 mg. was needed by M. G., and 75 mg. by L. M. The latter subject was not tested on an intake of 65 mg.

Blood levels. The amount of ascorbic acid found in fasting blood is believed by some to be the most accurate measure of the state of vitamin C nutrition. Here again there is a wide disagreement as to the level which represents tissue saturation. Faulkner and Taylor ('38) and Ralli et al. ('39) agree

that the renal threshold for ascorbic acid is about 1.3 to 1.4 mg. per 100 cc. It is at this blood level that the tissues are saturated; levels below this indicate varying degrees of unsaturation. On the other hand, there seems to be little agreement as to how low the blood levels may go without serious nutritional effects. Goldsmith and Ellinger ('39) and Wortis, Liebmann and Wortis ('38) believe 0.7 mg. per 100 cc. to be normal. The committee on vitamins of the American Academy of Pediatrics (Butler et al., '40) suggests that a

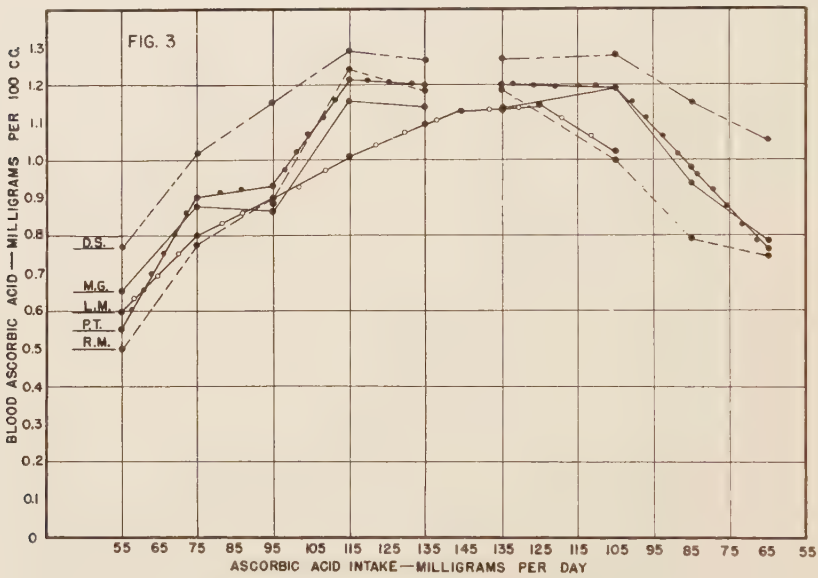

Fig. 3 Ascorbic acid concentration of the blood plasma of five subjects on varying levels of ascorbic acid intake.

serum or plasma concentration of 0.6 mg. or more per 100 cc. indicates a satisfactory state of vitamin C nutrition. Kajdi et al. ('39) believe that a blood level below 0.6 mg. per 100 cc. shows a serious depletion of body reserves. Whether these variations are due to differences in technique, or whether they are definite individual variations is difficult to determine.

At the beginning of the present study the blood levels of the children were between 0.5 and 0.6 mg. per 100 cc. The response of the blood plasma to graded amounts of vitamin C is illustrated in figure 3. The plasma values increased in

direct proportion to the intake until a plateau was reached at levels of 1.15 to 1.29 mg. per 100 cc. The lowest intakes which produced the maximum blood levels — the threshold value — were 105 mg. for three of the subjects, 115 mg. for the fourth, and 125 mg. for the fifth. As the intake was decreased below these amounts the plasma ascorbic acid likewise dropped.

To maintain blood values of approximately 0.9 mg. per 100 cc. the intake ranged from 65 to 95 mg. The two who required the highest intake to maintain this level, R. M. and L. M., were the ones who excreted the largest amount in response to the test dose. Also, their blood ascorbic acid rose more slowly than did that of the others.

On the lowest level of intake, 55 mg., the blood levels varied from 0.49 to 0.76 mg. per 100 cc. These individual variations in blood values were noted on all levels of intake. With adult subjects, Storvick and Hauck ('42) report variations even greater than these. If a blood value below 0.6 mg. per 100 cc. indicates a depletion of body reserves, then it would appear that the two largest subjects, P.T. and R.M., were depleted on the 55 mg. allowance. Only one subject, D. S., reached the plasma level of 0.7 mg. per 100 cc. on this intake. At least 65 mg. were required by all the others to bring the blood to 0.7 mg. per 100 cc., the concentration used in this study to indicate a satisfactory state of nutrition in respect to ascorbic acid. These results are in close agreement with those of Shelby ('41). In a study of thirty children, 6 to 12 years of age, she found that from 52 to 72 mg. were needed to maintain blood levels above 0.7 mg. per 100 cc. On the other hand, Bessey and White ('42) report that about 80% of their children had blood levels above 0.7 mg. per 100 cc. on intakes of 45 mg. This amount of ascorbic acid was estimated, however, on the consumption of citrus fruit and tomatoes only. Their results could better be compared then with the supplement alone used in the present study, and on this basis the results agree very well.

Of special interest are the blood levels corresponding to the "saturation" level. When a 50% excretion of the test dose

in 24 hours was used as the criterion for judging saturation, the blood ascorbic acid of the five subjects ranged from 0.60 to 1.05 mg. per 100 cc. These values are all below the renal threshold. In fact, some are below the levels suggested by some workers as being necessary for good nutrition.

COMPARISON OF REQUIREMENTS ON THE BASIS OF DIFFERENT METHODS OF ANALYSIS

The requirements as obtained by the several methods used in the interpretation of the data are summarized in table 4.

TABLE 4

Ascorbic acid requirements of five children as based upon various criteria for judging adequacy.

CRITERIA USED FOR JUDGING ADEQUACY	SUBJECT					
	M. G.	P. T.	D. S.	R. M.	L. M.	Average
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Intake to maintain average "retention" value [1]	75	75	75	55	55	67
Intake to maintain "resting level" of 40 mg. urinary excretion	85	85	85	85	75	83
Intake to insure "saturation"						
a. As indicated by 50% excretion of a 300-mg. test dose [1]	65	75	75	65	55	69
b. As indicated by method of Belser, Hauck, Storvick	95	75	75	65	75	77
Intake to maintain blood levels						
a. Of 0.7 mg. or above [1]	65	65	55	65	65	63
b. Of 0.9 mg. or above	75	75	65	95	95	81
c. Of maximum value	105	105	105	115	125	109

[1] The requirements for our subjects have been based upon these three indices.

Individually and collectively there is a wide range in the results. Including all methods of interpretation used, 65 mg. was the least value obtained for the requirements of the two oldest children, M. G. and P. T. Their blood levels were maintained above 0.7 mg. per 100 cc. at this level, and M. G. excreted 50% of the test dose in 24 hours. On all other bases, however, their requirements were higher. With the three

youngest children, 55 mg. was the least required. This was the lowest level providing maximum "retention" for two of the subjects. One of these, L. M., also excreted 50% of the test dose on this amount. Subject D. S. was the only child, however, who maintained a blood level above 0.7 mg. per 100 cc. when receiving 55 mg.

On the basis of the different criteria used it appears that an intake of 65 mg. for the two youngest children, and 75 mg. for the three oldest, would be sufficient to (1) maintain a blood level above 0.7 mg. per 100 cc., (2) provide for the maintenance of the average "retention" value and (3) insure saturation on the basis of a 50% excretion of a 300-mg. test dose. This represents a range of 1.7 to 2.4 mg. per kilogram of body weight. With other means of interpretation the requirements would be higher. Seventy-five to 85 mg., for instance, would be necessary to allow for a "resting level" of 40 mg. Amounts up to 95 mg. are needed to maintain blood levels of 0.9 mg. per 100 cc. In order to reach the threshold values these children required from 105 to 125 mg. of ascorbic acid.

SUMMARY

The vitamin C requirements of five children between the ages of 7 and 12 years were studied. Blood values and urinary excretion of ascorbic acid were determined on levels of intake ranging from 55 to 145 mg. Following each level of intake a 300-mg. test dose was given to determine the state of tissue saturation. The amount "retained" by the body, the blood concentration, and the response to the test dose were used as criteria for judging the nutrition of each subject in respect to vitamin C.

Sixty-five milligrams of ascorbic acid were required by the two youngest children, and 75 mg. by the three oldest, to promote saturation on the basis of a 50% excretion of the test dose in 24 hours. These amounts were sufficient, also, to maintain blood levels above 0.7 mg. per 100 cc., and to allow for average "retention." With other methods of interpretation the requirements varied from these amounts up to 125 mg.

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